

AMENDMENTS TO THE CLAIMS

Please amend claims 1 and 22, as shown in the following listing of claims, which will replace all prior versions and listings of claims in the application. Claims 1-5 and 22 are pending in the application.

Listing of claims:

1 (currently amended). A method to prepare an isolated nucleic acid fusion molecule having a nucleotide sequence encoding ~~at least one of each of the variable regions~~region of the ~~a T-cell receptor (TCR) α and β chain~~ fused to the variable region of a TCR β chain, ~~s of a non-human T-cell receptor (TCR) which TCR is said TCR α and TCR β chains comprising a non-human TCR specific for a tumor-associated antigen (TAA) and for HLA restriction~~restricted by HLA, which method comprises

- a. immunizing a transgenic non-human mammal species, which produces human HLA, with an effective amount of said TAA to produce HLA restricted cytotoxic T lymphocytes (CTL) ~~which display TCR specific for said TAA in amounts sufficient to lyse tumor cells having the TAA,~~
 - i. wherein the HLA restricted CTL comprise:
 - a TCR α chain nucleic acid molecule comprising a nucleic acid sequence encoding the variable region of a TCR α chain; and
 - a TCR β chain nucleic acid molecule comprising a nucleic acid sequence encoding the variable region of a TCR β chain; and
 - ii. wherein the HLA restricted CTL display a TCR that is comprised of the TCR α chain and the TCR β chain, specific for said TAA and displayed in amounts sufficient to lyse tumor cells having said TAA;
- b. recovering ~~said the~~ HLA restricted CTL ~~of step a, which contain a nucleic acid molecule comprising a nucleic acid sequence of a variable region of the α chain of the TCR and a nucleic acid sequence of a variable region of the β chain of the TCR;~~

c. ~~cloning or amplifying said nucleic acid molecule comprising nucleotide sequence isolated the TCR α chain nucleic acid molecule and the TCR β chain nucleic acid molecule from the HLA restricted CTL and encoding a variable region of the α chain of the TCR and a variable region of the β chain of the TCR to provide TCR receptor encoding nucleic acid molecules comprising at least one nucleic acid molecule, which comprises a sequence encoding a variable TCR α chain protein, and at least one nucleic acid molecule, which comprises a sequence encoding a variable TCR β chain protein of step b to provide a TCR α chain nucleic acid product comprising a nucleic acid sequence encoding the variable region of the TCR α chain and a TCR β chain nucleic acid product comprising a nucleic acid sequence encoding the variable region of the TCR β chain, respectively;~~

d. ~~recovering said TCR receptor encoding nucleic acid molecules~~the TCR α chain nucleic acid product and the TCR β chain nucleic acid product of step c; and

e. ~~fusing the recovered TCR receptor encoding nucleic acid molecules together~~ α chain nucleic acid product to the recovered TCR β chain nucleic acid product to prepare the isolated fused nucleic acid molecule, wherein the fused nucleic acid molecule comprises a sequence encoding a single-chain TCR comprising a fusion protein, which comprises a variable region of the TCR α chain fused to a variable region of the TCR β chain and which is specific for TAA and for HLA restriction.

2 (previously presented). The method of claim 1 wherein said HLA antigen is A2.

3 (previously presented). The method of claim 1 wherein said mammal is a mouse.

4 (previously presented). The method of claim 3 wherein the cloning or amplifying step c comprises a polymerase chain reaction using primers derived from murine TCR.

5 (previously presented). The method of claim 4 wherein said primers are set forth in Figure 6 (SEQ ID NOS: 3-42).

6-21 (canceled).

22 (currently amended). The method of claim 1, wherein the ~~variable region of the~~
~~TCR α chain of step e comprises a functional variable region of the TCR α chain and the variable~~
~~region of the TCR β chain of step e comprises a functional variable region of the TCR β chain~~
single chain TCR of step e comprises a TCR derivative that retains the HLA restriction and
TAA-specificity characteristics of the TCR of step a.